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Effects of a Hen's Egg Yolk Diet on Certain Inorganic Elements in the Snail *Helisoma trivolvis* (Colorado Strain)

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Abstract. Graphite furnace atomic absorption spectrometry, flame atomic absorption spectrometry, and ion chromatography were used to investigate several elements in the whole body, digestive gland-gonad complex (DGG), shell, and plasma of the pulmonate snail, *Helisoma trivolvis* (Colorado strain), maintained in artificial spring water (ASW) on two different diets, hen's egg yolk (Y) and Romaine leaf lettuce (L). Whole body and DGG samples were analyzed for the following seven elements: sodium, potassium, calcium, magnesium, zinc, iron, and manganese. Of these, iron was present in a significantly higher concentration (Student's *t*-test, $P < 0.05$) in the whole bodies of snails on the L-diet compared to those on the Y-diet. For the DGG analysis, calcium and potassium were present at significantly higher concentrations, and magnesium at a significantly lower concentration, in snails on the L-diet. Plasma was analyzed for calcium and iron, and no significant differences were found in the concentrations of these elements in snails on both diets. The shells of *H. trivolvis,* analyzed only for calcium, showed no statistical difference in the concentration of this element between snails on the L-diet versus those on the Y-diet. The Romaine leaf lettuce, the hen's egg yolk, and the ASW were analyzed for certain elements: food samples for potassium, magnesium, calcium and iron, and ASW for calcium and iron. There were significantly higher concentrations of calcium and iron in the hen's egg yolk compared to the Romaine leaf lettuce (Student's *t*-test, $P < 0.05$). The ASW contained calcium at a concentration of 20.0 ± 0.0 mg L^{-1}, and a trace amount of iron at 0.0307 ± 0.017 mg L^{-1}. The occurrence of certain elements in the snail may be considered as the ultimate result of passage of these elements from the lettuce or egg yolk upon which the snails were fed or the water in which they were maintained.

INTRODUCTION

Helisoma trivolvis (Say, 1816) is a ubiquitous fresh water planorbid snail in North America. Numerous strains of this snail have been reported, but the taxonomic relationships within the species are still uncertain. One of us (BF) has maintained two strains of *H. trivolvis* in the laboratory for a number of years. One strain, *H. trivolvis* (Pennsylvania strain), is heavily pigmented with melanin and has a black body. This strain serves as a vector of the 37-collar-spined echinostome, *Echinostoma trivolvis* (Cort, 1914), in the USA (see Huffman & Fried, 1990 for a review). The second strain, *H. trivolvis* (Colorado strain), is refractory to infection with *E. trivolvis,* lacks melanin, and has an orange-red body. The Colorado strain has been used extensively in neurobiology studies (see Kater, 1974).

Two of us (BF and JS) used a high fat diet (hen's egg yolk) in the late 1980s to induce hyperlipidemia and hyperlipemia in the medically important planorbid snail *Biomphalaria glabrata* (Say, 1816) (see reviews in Fried & Sherma, 1990, 1993). In addition to studies on neutral and polar lipids in the snails maintained on the high fat diets, recent work examined carbohydrates (Kim et al., 2001) and lipophilic pigments (Kim et al., 2002; Evans et al., 2004) in such snails. Recently, our laboratory has examined the lipid composition of *H. trivolvis* (Co) in snails maintained on a hen's egg yolk diet. As expected, both juvenile and adult snails maintained on this diet accumulated significant amounts of certain lipids compared to cohorts maintained on a lettuce leaf diet (Schneck et al., 2003a, b).

There are no studies on elements in snails raised on

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various diets, and the present study was designed to determine the effects of a high fat diet (hen's egg yolk) on the element composition of *H. trivolvis* (Co) snails. Elements in the snails raised on the high fat diet were compared with those of snails maintained on a low fat diet (Romaine lettuce leaf). Previous studies have examined certain elements in snails independent of diet or infection with larval trematodes. Some of these representative studies and their findings are the following: Layman et al. (1996a) used atomic absorption spectrometry (AAS) and inductively coupled plasma-atomic emission spectrometry (ICP-AES) to study metal ions in the DGGs of *H. trivolvis* (PA) infected with *E. trivolvis* and uninfected *H. trivolvis* snails. They found sodium present in significantly higher amounts, and magnesium and manganese in significantly lower amounts, in the infected versus uninfected DGGs. Layman et al. (1996b) measured metallic ions in *B. glabrata* snails infected with *E. caproni* and in uninfected snails by ICP-AES and found no significant differences (Student's *t*-test, $P > 0.05$) in the concentrations of the metals in whole infected versus whole uninfected snails. Kaufer et al. (2002) analyzed the effects of *Euhaplorchis californiensis* Martin, 1950 infection on the metal ion concentrations in the DGGs of the marine snail *Ceritlhidea californica* Haldeman, 1840 by graphite furnace atomic absorption spectrometry (GFAAS) and ion chromatography (IC) and reported calcium present in significantly higher amounts, and magnesium in significantly lower amounts, in infected versus uninfected DGGs. Ong et al. (2004) investigated the effects of *Schistosoma mansoni* Sambon, 1907 infection on inorganic elements in the whole bodies of *B. glabrata* snails and reported significantly higher amounts of calcium, cadmium, manganese, and sodium in the whole infected versus whole uninfected snails.

Because there are no previous studies on the effects of a high fat diet on the element content of planorbid snails, the purpose of this study was to examine certain elements in *H. trivolvis* (Co) maintained on a hen's egg yolk diet. Controls consisted of cohort snails maintained on a diet of Romaine leaf lettuce.

MATERIALS AND METHODS

Snail Maintenance

Stock cultures of *H. trivolvis* (Co) were maintained from eggs to sexually mature adults at 23 ± 1°C in aerated glass jars, each containing 10 to 20 snails in 800 mL of artificial spring water (ASW) (Schneck et al., 2003a). The ASW was prepared as described by Ulmer (1970). One culture of 25 snails with shell lengths ranging from 16–20 mm was maintained *ad libitum* on boiled Romaine lettuce leaf (L-diet) for 20 weeks. Another culture of 25 snails (16–20 mm shell length) was first maintained on the L-diet for 16 weeks and then on the boiled hen's egg yolk diet (Y-diet) for an additional four weeks. For all the cultures, food and water were changed twice a week.

Sample Preparation

All glassware used for sample preparation and element analysis was cleaned as follows: first washed with soap and rinsed with tap water, soaked in 10% nitric acid solution for at least 2 hr, rinsed with deionized water at least three times, and finally dried in an oven overnight at 250°C. These steps were performed to ensure removal of any elements adhering to glass surfaces. Trace metal grade nitric acid (Fisher Scientific, Fair Lawn, New Jersey) was used in all experiments throughout this research.

Whole Body, Digestive Gland-Gonad Complex (DGG), and Shell

The whole bodies, DGGs, and shells of 10 individual snails (n = 10) on each diet (lettuce or yolk) were prepared as described below. During sample preparation, all snail dissections were performed in 6 cm diameter petri dishes. The shells of the snails were gently cracked with a blunt forceps, and the snail bodies removed from the shells and weighed (range 50 to 250 mg blotted wet weight). Shell samples were collected at the same time whole body samples were prepared and were obtained as follows: the shell pieces remaining in the petri dish after removal of the snail bodies as described above were collected with a forceps and weighed (range 30 to 120 mg blotted wet weight). For DGG samples, snail bodies were first obtained as described above. Each DGG was then dissected free of the visceral mass under a dissecting microscope with forceps and weighed (range 51 to 100 mg blotted wet weight). The visceral masses were discarded. All samples were rinsed several times with deionized water, and kept moist in 6 cm diameter petri dishes lined with filter paper that had been previously dampened with deionized water. Prior to analysis, each sample (whole body, DGG, or shell) was digested in 2 mL of boiling concentrated nitric acid in a 10 mL beaker. Each digested sample was diluted to 10.00 mL in a volumetric flask with 2% (v/v) nitric acid.

Whole body and DGG samples were analyzed for the following elements: calcium, iron, potassium, magnesium, sodium, manganese, and zinc. Calcium, potassium, magnesium, and sodium were determined by FAAS, and iron, manganese, and zinc by GFAAS. Shell samples were analyzed for calcium by FAAS. To ensure that sample concentrations were within the range of the calibration curve of the standards, the shell samples were diluted 2000-fold with 2% (v/v) nitric acid. For all calcium analyses, a lanthanum (III) nitrate solution [31 g lanthanum (III) nitrate hexahydrate salt diluted in 100 mL 2% (v/v) nitric acid] was added in an amount that was equivalent to 10% of the flask size to remove interfering phosphate ions that prevent the volatilization of calcium. For sodium

analysis, potassium chloride [4 g potassium chloride diluted in 100 mL 2% (v/v) nitric acid] was added in a volume that was 10% of the flask size to suppress ionization of sodium atoms.

Plasma

An additional 20 snails were used to prepare the plasma samples (10 snails each on the L- and Y-diets). Samples of plasma, which is defined as hemolymph minus the hemocyte fraction, were obtained as follows: to obtain hemolymph, snail shells were cracked open gently with a blunt forceps and the hemolymph allowed to ooze out into a dry petri dish. The hemolymph was removed from the petri dish using a Pasteur pipet and pooled in Eppendorf tubes. Hemolymph from three or four snails on the lettuce diet was pooled to prepare a sample of approximately 300 μL. Likewise, hemolymph was pooled from three or four snails on the yolk diet to prepare an approximate 250 μL sample from that population. For each diet, three pools of the hemolymph (n = 3) were prepared from 10 snails.

The hemolymph samples were centrifuged at 5600 × g for 5 min to obtain a pellet (consisting of hemocytes and some residual snail debris) that was discarded, and the supernatant (plasma) was used. The plasma was transferred to a new Eppendorf tube using a Pasteur pipet. The sample was then diluted 10-fold in 0.01 M nitric acid and analyzed for calcium by IC and for iron by GFAAS.

Snail Food

Romaine lettuce and hen's eggs (domestic chickens) were boiled for approximately 10 min prior to use. Only the green, leafy portion of the lettuce and the yolk of the egg were used for analyses. Both foods were blotted dry prior to weighing. Approximately 5 g of lettuce and 1 g of yolk were weighed accurately in separate beakers, and three samples were prepared for each diet (n = 3). The lettuce sample was digested in 37 mL of boiling concentrated nitric acid in a 25 mL beaker until a thin yellow film remained at the bottom of the beaker. The yolk was digested in 32 mL of boiling concentrated nitric acid in a 25 mL beaker. Concentrated sulfuric acid (5 mL) was added to the digest to aid in raising the temperature of the solution. A dark colored film remained at the end of the yolk digestion. For both diets, the resulting film was dissolved in 10 mL in a volumetric flask with 2% (v/v) nitric acid. The lettuce and yolk were analyzed for calcium, iron, potassium, and magnesium by FAAS, and for iron by GFAAS.

Artificial Spring Water (ASW)

Three samples of ASW (n = 3) were collected and analyzed for calcium and iron by FAAS and GFAAS, respectively. The ASW samples were diluted 100-fold for calcium determination and 2-fold for iron determination using 2% (v/v) nitric acid.

Elemental Analysis by Atomic Absorption Spectrometry (AAS) and Ion Chromatography (IC)

GFAAS was utilized to quantify the levels of iron, manganese, and zinc, while FAAS was used to analyze for calcium, potassium, magnesium, and sodium. Calcium determination in the plasma samples was performed by IC because sample volumes were too small to allow the use of FAAS.

The GFAAS instrument was a GBC 932 plus (GBC Scientific Equipment, Arlington Heights, Illinois) atomic absorption spectrometer with GBC GF3000 graphite furnace system, separate hollow cathode lamps (Varian, Inc., Walnut Creek, California) for each element determined, GBC PAL3000 autosampler, and GBC Advanta version 1.33 software. The instrument had a double beam design and a deuterium background correction system. All standard and sample volumes were 20 μL. Stock standard solutions of each metallic ion in 2% HNO_3 were made and autodiluted with 2% HNO_3 into multiple working standards by the instrument. The lamp current and wavelength, slit width, and oven temperature program were optimized for each element. All samples were analyzed in triplicate to obtain mean absorbance values. The instrument provided the experimental concentration of each test solution by interpolation from the calibration curve (mean absorbance versus the working range of the element: 100–2000 μg L^{-1} for Fe and Zn, 25–300 μg L^{-1} for Mn).

The FAAS instrument was a Varian SpectrAA-20 atomic absorption spectrometer (Varian, Inc.) with a 1-lamp turret arrangement, separate hollow cathode lamps (Varian Techtron) for each element determined, and an air-acetylene burner. The instrument had a double-beam design and no background-correction system. Wavelength settings, slit selection, lamp current, and gas flows were optimized for each element. Five standard solutions were prepared for analysis of each element with the following working ranges (mg L^{-1}): Ca 0.20–5.0, Mg 1.0–50, Na 0.040–2.0, K 1.0–50. The standards and samples were analyzed using three 30 s integrations. The instrument provided the experimental concentration of each test solution by interpolation from the calibration curve (mean absorbance versus element concentration).

The IC instrument was a DX-120 ion chromatograph (Dionex, Sunnyvale, California) with an AS40 automated sampler, IonPac CG12A guard column (4 × 50 mm), IonPac CS12A cation exchange analytical column functionalized with weak phosphonic and carboxylic acid groups (4 × 250 mm), and self-regenerating membrane cation suppressor system. The isocratic mobile phase was 20 mM methanesulfonic acid at a flow rate of 0.98 mL/

Table 1

Mean concentration ± standard deviation in mg g^{-1} of wet tissue of whole bodies obtained by FAAS and GFAAS for elements in *H. trivolvis* maintained on the L- and Y-diets.

Element	L-diet[a]	Y-diet[a]	Value of *P*
Ca	5.43 ± 2.8	4.26 ± 1.8	0.292
Fe	0.0187 ± 0.0060	0.0112 ± 0.0036	0.00409[b]
K	1.34 ± 0.34	1.27 ± 0.20	0.628
Mg	0.863 ± 0.25	0.863 ± 0.22	0.998
Mn	0.00632 ± 0.0018	0.00470 ± 0.0032	0.195
Na	0.273 ± 0.054	0.324 ± 0.15	0.331
Zn	0.0647 ± 0.061	0.0446 ± 0.024	0.348

[a] n = 10 samples, where each sample consisted of the whole body of an individual snail.

[b] Differences significant at $P < 0.05$ as determined by the Student's *t*-test.

Table 2

Mean concentration ± standard deviation in mg g^{-1} of wet tissue of the DGGs obtained by FAAS and GFAAS for elements in *H. trivolvis* snails maintained on the L- and Y-diets.

Element	L-diet[a]	Y-diet[a]	Value of *P*
Ca	2.44 ± 0.57	1.68 ± 0.41	0.00329[b]
Fe	0.0406 ± 0.019	0.0234 ± 0.019	0.0602
K	0.927 ± 0.32	0.669 ± 0.18	0.0414[b]
Mg	0.498 ± 0.096	0.648 ± 0.14	0.0119[b]
Mn	0.00185 ± 0.00059	0.00178 ± 0.00056	0.792
Na	0.425 ± 0.14	0.343 ± 0.078	0.122
Zn	0.0254 ± 0.015	0.0431 ± 0.029	0.0829

[a] n = 10 samples, where each sample consisted of the DGG of an individual snail.

[b] Differences significant at $P < 0.05$ as determined by the Student's *t*-test.

min. Three standard calcium solutions with concentrations of 5, 50, and 100 mg L^{-1} were prepared in 0.01 M nitric acid for generation of a linear least-squares calibration curve. The calibration curves and interpolated sample concentrations were obtained using PeakNet Chromatography Workstation software. The injection volumes of standards and samples were 25 µL, and all solutions were analyzed in triplicate.

Data Analysis

For all the three analytical methods (GFAAS, FAAS, and IC), the measured element concentration in the test sample solutions was provided by the instrument by interpolation of bracketed samples from the calibration curve. For the whole body, DGG, shell, and diet samples, the concentration of each element (mg g^{-1}) in the samples was calculated using the following equation:

$$\text{concentration of element} = \frac{(C)(V)(D)}{(M)(1000)} \qquad (1)$$

where C is the test solution concentration from the instrument (mg L^{-1}), V is the volume of the initial dilution of the sample following digestion (10 mL), D is the appropriate dilution factor made for each analysis, and M is the mass of the wet sample (g). The mean concentrations of the elements in the samples obtained from the lettuce fed snails versus the yolk fed snails were statistically compared with the Student's *t*-test (two-sample assuming unequal variances) in Microsoft Excel 2000.

For the plasma and ASW samples, the concentration of the elements (mg L^{-1}) determined were calculated with the following equation:

$$\text{Concentration of element} = (C)(D) \qquad (2)$$

where C is the test solution concentration provided by the instrument (mg L^{-1}), and D is the dilution factor (plasma 10; ASW iron 2, calcium 100). Again, Microsoft Excel 2000 was used to compare for statistical differences in the mean concentrations of the elements in the plasma samples of the snails on the two different diets using the Student's *t*-test ($P < 0.05$).

RESULTS

The seven elements determined in the whole bodies of snails maintained on both diets were present in the following concentration order: calcium > potassium > magnesium > sodium > zinc > iron > manganese. The concentrations of these elements were elevated in snails on the L-diet, with the exception of magnesium, which was present in equal concentrations for snails on both diets, and sodium, which was at a higher concentration in snails on the Y-diet. Iron was the only element with a significantly higher concentration in the whole bodies of snails on the L-diet compared to the snails on the Y-diet (Student's *t*-test, $P < 0.05$). Table 1 lists quantitative data for the snail mass-adjusted concentrations of the elements, calculated using Equation 1, for the whole bodies of *H. trivolvis* (Co) on both diets. The same seven elements were quantified in the DGGs. Table 2 lists quantitative data for the snail mass-adjusted (calculated using Equation 1) element concentrations in the DGGs of *H. trivolvis* (Co) snails on both the L- and Y-diets. For DGGs of snails on the Y-diet, the elements were present in a concentration order similar to the one in the whole bodies of the snails described above. The DGGs of the snails maintained on the L-diet showed the following order in concentration of the elements: calcium > potassium > magnesium > sodium > iron > zinc > manganese. These elements were all present at higher concentrations in DGGs of snails on the lettuce diet with the exception of magnesium and zinc, which were at lower concentrations compared to DGGs of snails on the Y-diet. There was a

Table 3

Mean concentration ± standard deviation in mg g^{-1} obtained by FAAS and GFAAS for the wet weights of Romaine leaf lettuce and hen's egg yolk.

Element	Lettuce[a]	Egg yolk[a]	Value of *P*
Ca	0.989 ± 0.23	1.85 ± 0.27	0.0138[b]
Fe	0.00468 ± 0.0012	0.0148 ± 0.0026	0.00839[b]
K	1.78 ± 0.64	1.27 ± 0.063	0.303
Mg	0.252 ± 0.073	0.287 ± 0.024	0.509

[a] n = 3 samples, where the lettuce samples were approximately 5 g each, and the egg yolk samples were approximately 1 g each.
[b] Differences significant at $P < 0.05$ as determined by the Student's *t*-test.

significantly lower concentration of calcium and potassium, and a significantly higher concentration of magnesium, in the DGGs of snails maintained on the egg yolk diet than those maintained on the lettuce diet (Student's *t*-test, $P < 0.05$).

The concentrations of calcium (calculated using Equation 1) in the shells of *H. trivolvis* (Co) on the L- and Y-diets were 389 ± 54 mg g^{-1} and 378 ± 62 mg g^{-1}, respectively, and were not significantly different (Student's *t*-test, $P > 0.05$). The plasma samples, analyzed only for calcium and iron, gave the following results (calculated using Equation 2): 278 ± 43 mg L^{-1} and 233 ± 9.7 mg L^{-1} in snails on the L- and Y-diet, respectively, for the calcium analysis; 10.3 ± 1.2 mg L^{-1} and 10.1 ± 1.6 mg L^{-1} in snails on the L- and Y-diet, respectively, for the iron analysis. No significant differences were found between the concentrations of both elements in the plasma of snails maintained on both the L- and Y-diet (Student's *t*-test, $P > 0.05$).

The results obtained for certain elements in Romaine lettuce leaf and hen's egg yolk are summarized in Table 3. These foods were analyzed for the elements calcium, iron, potassium, and magnesium. Among these four elements determined, the calcium and iron concentrations in the hen's egg yolk were significantly higher than those found in the lettuce (Student's *t*-test, $P < 0.05$).

The results for the calcium and iron determination in the ASW (calculated using Equation 2) were 20.0 ± 0.0 mg L^{-1} and 0.0307 ± 0.017 mg L^{-1} for calcium and iron, respectively. According to the standard water hardness classification of the United States Geological Survey, this calcium concentration indicates that the ASW is of a slightly hard level.

DISCUSSION

The elements that were analyzed in this investigation were selected for several reasons. First, potassium, magnesium, sodium, and calcium are among the elements known to be of highest concentrations in many biological systems (Prosser, 1973). These elements have diverse functions in animal cells and are necessary for normal cellular functions. For instance, calcium, potassium, and magnesium are important for muscle contraction and nerve cell function. The construction of skeletal and shell components is dependent on calcium and magnesium. Magnesium is also an essential cofactor for some enzymes, including the ATPases and kinases (Prosser, 1973). Iron, present in the heme portion of the oxygen carrier hemoglobin, was selected based on visual observations during laboratory work that the colors of the hemolymph of the snails maintained on the lettuce versus yolk diets were different. Snails fed the lettuce diet had hemolymph that was bright red while snails fed the yolk diet had hemolymph that was yellow-orange. The heavy metals zinc and manganese are necessary in trace amounts in biological systems, but are toxic in high concentrations (Prosser, 1973). Zinc and manganese both act as cofactors of certain enzymes found in living systems.

The results obtained are similar to those of Kalyani (2001), who examined certain elements in the giant African land snail, *Achatina fulica* Bowdich, 1822, and estimated calcium to be the major element in both the soft body and shell, followed by potassium, magnesium, and sodium. We found the same order in the concentrations of these elements in the whole bodies and DGGs of *H. trivolvis* (Co) on the L- and Y-diets.

The whole body analysis of *H. trivolvis* (Co) included the major regions of the snail, i.e., the head-foot, viscera, and DGG. The significant depletion of iron in the snail whole bodies on the Y-diet, compared to snails on the L-diet, possibly reflects the presence of blood sinuses in the viscera and head-foot regions of yolk-fed snails, which contain low iron content in the hemolymph and tissue.

The snail DGG (particularly the digestive gland portion) is the main site of interest in dietary studies because it is a good indicator of snail metabolic activity. The DGG contains the digestive gland or hepato-pancreas (liver) and the reproductive glands of the snail, the ovotestis. A major function of DGG cells is the storage of various metals held in membrane-insoluble granules in the cells (Howard et al., 1981; Simkiss, 1981; Dallinger & Wieser, 1984; Bebianno & Langston, 1995). Furthermore, the DGG is an important target site for most metabolic and enzymatic activities (Dallinger & Wieser, 1984; Bebianno & Langston, 1995). In our study, calcium and potassium were significantly higher, and magnesium significantly lower, in the DGGs of snails on the L-diet compared to the Y-diet. The DGGs of the yolk-fed snails were yellow-white, in contrast to the DGGs of the lettuce-fed snails, which were dark green-brown. Schneck et al. (2003a) and Evans et al. (2004) made similar observations on the gross appearance of DGGs from snails on the two diets. This color difference may reflect an increased deposition of fat in the DGGs of the yolk-fed

snails, and a subsequent alteration in the element concentrations.

Red blood, due to hemoglobin dissolved in the plasma, is characteristic of planorbid snails. We analyzed iron in the plasma to see if there were differences in the concentration of this element in the snails on the two diets. Schneck et al. (2003a) reported that snails on the L-diet yielded more hemolymph compared to the Y-diet (100 μL and 50 μL per snail, respectively). Furthermore, snails fed the L-diet had a red hemolymph compared to the yellow-orange color in snails on the Y-diet. These observations suggested that the hemoglobin content, and perhaps the iron content, was different in both snail populations. However, the iron concentration was not significantly lower in the yolk-fed snails as compared to the lettuce-fed snails (Student's *t*-test, $P > 0.05$). The color difference in the plasma of the snails probably reflects the presence of lipophilic pigments, i.e., carotenes and xanthophylls, in the plasma of snails maintained on the yolk diet.

The shells of *H. trivolvis* (Co) were analyzed only for calcium, the chief constituent of shells. The calcium concentrations in the shells of snails fed the L- and Y-diets were determined to be 389 ± 54 mg g^{-1} and 378 ± 62 mg g^{-1}, respectively. According to Marxen et al. (2003), the constituents of the molluscan shell are calcium carbonate, present in a concentration of 95 to 99.9%, and organic material, 0.1 to 5%. Calcium carbonate is the insoluble product of the two major inorganic ions in pulmonate shells, calcium and bicarbonate; both ions are obtained from the animal's nutrients and the environment, with bicarbonate being additionally drawn from the animal's metabolic production of carbon dioxide (Luchtel et al., 1997). Calcium carbonate is usually present in one of two predominate crystalline lattice configurations in the pulmonate shell, aragonite or calcite, and sometimes vaterite (Luchtel et al., 1997). Assuming that all of the calcium present in the shell is in the form of calcium carbonate, the percentage of calcium carbonate in the *H. trivolvis* shell obtained in this study is equivalent to 94% and 97% in the lettuce- and yolk-fed snails, respectively.

Schneck et al. (2003a) observed that the shells of *H. trivolvis* (Co) snails maintained on the Y-diet were more fragile and, therefore, more susceptible to cracking than those on the L-diet, suggesting that snails on the Y-diet were lacking calcium in their shells. We found no significant difference in calcium concentration in shells from snails on both diets; hence, shell fragility must be due to factors other than the calcium content of the shell.

Romanoff & Romanoff (1949) reported that the most abundant element in egg yolk is phosphorus, accounting for 0.588% of the yolk's mass. Other inorganic elementals found in egg yolk in small quantities, and their percentages, included calcium (0.144%), magnesium (0.128%), chlorine (0.123%), potassium (0.112%), and sodium (0.070%). Iron and sulfur were present in trace amounts of 0.011% and 0.016% in yolk, respectively. We found that the order of the elements in our egg yolk samples was calcium > potassium > magnesium > iron. The magnesium level in hen's egg yolk in our study was lower than that reported by Romanoff & Romanoff (1949), and this may be attributed to a difference in the method of analysis (method not reported by Romanoff & Romanoff, 1949). Additionally, Romanoff & Romanoff (1949) stressed the important fact that the concentrations of elements in egg yolk, albumen, and shells are dependent upon the concentrations of elements in the diets fed to the hens.

We found no literature on inorganic elements present in Romaine lettuce. Our findings on the elements in Romaine lettuce appear to be the first ever reported. The ASW of Ulmer (1970) is widely used by malacologists and parasitologists to maintain planorbid snails. Our quantitative results on calcium and iron concentrations in this water may be of interest to these workers. The occurrence of certain elements in the snail tissue, plasma and shell may be considered as the ultimate result of passage of these elements from the lettuce or hen's egg yolk upon which the snails were fed, or the water in which they were maintained.

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